

apparent opposing effects of PRMT5 activity on tumorigenesis need further investigation.

Fourth, the observation of increased phosphorylation of PRMT5 in CD34+ cells from one MPN patient without activating JAK2 mutation suggests that other tyrosine kinases could phosphorylate PRMT5. Will impaired PRMT5 also be the consequence of uncontrolled activity of tyrosine kinases, such as mutated ABL, PDGFR, or FLT3, frequently found in chronic and acute malignant myeloproliferations?

Finally, since phosphorylation and inhibition of PRMT5 activity are properties specific for the mutated JAK2 proteins, inhibiting the interaction between mutant JAK2 and PRMT5 could potentially inter-

fere with MPN pathogenesis without affecting the wild-type JAK2.

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Monoallelic Deletion of *NFKBIA* in Glioblastoma: When Less Is More

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Bredel et al. (2010) recently identified a subset of glioblastomas that harbor monoallelic loss of *NFKBIA*, which negatively affects patient prognosis. This finding raises new questions as to the role of I κ B α and NF- κ B in glioblastoma, the relationship between EGFR and NF- κ B signaling, and potential therapeutic targets.

When we think of mutations that promote cancer, those used for textbook and review examples, the NF- κ B pathway does not get a lot of attention. Yet NF- κ B is clearly established as an important mediator of oncogenesis (Karin, 2006), and mutations in the immediate regulatory pathways leading to NF- κ B activation have been characterized (Courtois and Gilmore, 2006). Importantly, well-established mutations that lead to cancer, such as activating mutations in Ras, function oncogenically through NF- κ B activation (Bassères et al., 2010; Mayo et al., 1997; Meylan et al., 2009). New work from Bredel et al. (2010) demonstrates a fascinating monoallelic

deletion of *NFKBIA*, in patient-derived glioblastoma multiforme (GBM). This gene encodes I κ B α , a critical negative regulator of canonical NF- κ B activation. The work suggests an interesting link between EGFR-induced signaling in GBM and the loss of I κ B α .

NF- κ B is a transcription factor comprised of homo- and heterodimers of five subunits: p65/RelA, RelB, c-Rel, p105/p50, and p100/p52. Under basal conditions, I κ B molecules (I κ B α , β , and ϵ isoforms) sequester p65- and c-Rel-containing dimers in the cytoplasm. Full-length p100 and p105 contain similar motifs to I κ B and must be processed to yield active p50 and p52 subunits. Upon

activation by cytokines or other stimuli, the IKK complex phosphorylates I κ B, leading to its proteasomal degradation, which leaves NF- κ B free to accumulate in the nucleus to control target gene expression. Genes regulated by NF- κ B promote cell proliferation and survival, underlying the importance of this transcription factor both in normal cell responses and in oncogenesis (Karin, 2006).

Due to the integral involvement of NF- κ B in promoting proliferation and the survival of cells of the hematopoietic system, it is not surprising that mutations in this pathway are observed in hematologic malignancies. c-Rel is often amplified in B cell malignancies and Hodgkin's

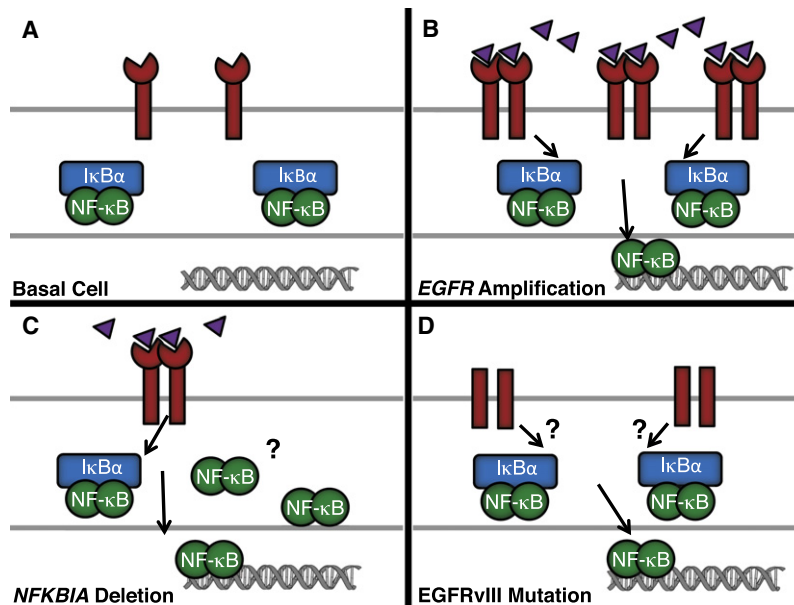


Figure 1. Link between EGFR and NF- κ B Pathways in Glioblastoma

(A) In a basal setting, neither EGFR nor NF- κ B is activated. (B) In a glioma cell with *EGFR* amplification, the overexpressed receptor is present on the membrane in dimers and responds to EGF, ultimately leading to NF- κ B activation. (C) In a glioma cell with *NFKBIA* deletion, normal EGFR is present, and stimulation by EGF is followed by NF- κ B signaling. In this setting, EGF signaling to NF- κ B is proposed to be facilitated by the loss of I κ B α , which may also lead to higher basal NF- κ B activity, free NF- κ B dimers in the cytoplasm or nucleus, or compensation by other I κ B isoforms. Additionally, the loss of I κ B α may negate a potential NF- κ B-independent mechanism for this inhibitor. (D) In a glioma cell with EGFRvIII expression, the cell constitutively signals through the EGFR pathway independent of EGF. It remains to be determined if EGFRvIII can activate NF- κ B in a similar manner to wild-type EGFR. Purple triangles represent EGF, while red receptors represent EGFR.

lymphoma. Presumably, the abundance of c-Rel subunits overcomes the inhibitory mechanism of the I κ Bs, leading to constitutive activation. In both B cell and T cell leukemias/lymphomas, p100 truncations eliminate the inhibitory domains but leave p52 intact, resulting in a weakly oncogenic NF- κ B subunit. The oncoprotein BCL-3, an I κ B-related NF- κ B coactivator that functions with the p50 and p52 subunits, is involved in the t(14:19) translocation in B cell CLL, ultimately increasing BCL-3 expression (Courtois and Gilmore, 2006). Analysis of multiple myeloma samples shows increased NF- κ B activity in the majority of patients, which occurs through alterations to a number of upstream NF- κ B signaling molecules including BIRC2/3, CYLD, CD40, NFKB1, NFKB2, NIK, and TRAF3 (Annunziata et al., 2007). NF- κ B-related mutations are not limited to leukemias. IKK ϵ is a kinase related to IKK α and β , typically involved in innate immune signaling, but it has also been shown to be capable of canonical NF- κ B pathway

activation. Amplification of the genomic locus leading to IKK ϵ overexpression has been documented in breast cancer cell lines and tumor samples (Boehm et al., 2007). Given the strong evidence of NF- κ B subunits exhibiting oncogenic activity, it follows that the inhibitory I κ Bs would demonstrate tumor suppressor function. Consistent with this, as many as 10% of Hodgkin's lymphoma specimens show inactivation of both alleles of *NFKBIA* (Courtois and Gilmore, 2006).

By analyzing a large cohort of human glioblastomas, Bredel et al. (2010) discovered heterozygous *NFKBIA* deletions in almost 25% of tumors. Interestingly, the loss of *NFKBIA* generally does not overlap with *EGFR* amplification, one of the most common genetic alterations in glioblastoma. In fact, EGFR-induced signaling is considered important for most, if not all, of glioma. Since it has been shown that EGFR can activate NF- κ B, this mutual exclusivity suggests that EGFR and I κ B α fall in the same pathway. Consistent with this idea, Bredel et al. (2010) show that

both *NFKBIA*-deleted tumors and *EGFR*-amplified tumors have a similar decrease in patient survival compared with those that are wild-type for both genes. They also demonstrate that expressing I κ B α in *NFKBIA*-deleted or *EGFR*-amplified primary cultures decreased their viability, while cells that were wild-type for both were unaffected. Work from the Cancer Genome Atlas project identified four distinct subtypes in glioma: classical, mesenchymal, neural, and proneural (Verhaak et al., 2010). When the distribution of these two mutations is analyzed based on tumor subtype, those with *EGFR* amplification tend to cluster as classical, while those with *NFKBIA* deletion tend to fall into the three nonclassical subtypes. One interpretation is both of these mutations may cause NF- κ B activation as a more general feature of GBM, regardless of subtype (Figure 1). In future work, novel mutations that affect this signaling axis to a similar end may be discovered in tumors that are wild-type for EGFR and I κ B α . On the other hand, these mutations may have substantially different effects on the signaling in the tumor, manifested by the distinct segregation into different subtypes. Further studies to directly compare *EGFR*-amplified samples with *NFKBIA*-deleted ones will be useful in understanding the common characteristics and the unique properties of these subsets.

While genomic *NFKBIA* deletion seems to be fairly common in GBM and has a significant impact on patient prognosis, there are several questions raised by this report that remain unanswered. First, the current study needs to be followed by functional validation in vitro and in vivo. As the level of NF- κ B activity was not assessed in the tumors, it remains to be determined if *NFKBIA* deletion affects the NF- κ B pathway. While it is known that glioma cell lines exhibit elevated levels of NF- κ B activity, the source of this activation remains unclear. Additionally, comparing levels of NF- κ B activity between tumors with *NFKBIA* deletion and *EGFR* amplification will help to determine if these two mutations produce a phenotypically similar disease. One particular endpoint to assess would be IL-6, a known NF- κ B target gene. EGFRvIII, a constitutively active mutant EGFR, has been shown to increase IL-6 levels, which can act in a paracrine manner to

activate wild-type EGFR on other tumor cells (Inda et al., 2010). One could imagine that loss of I κ B α in EGFR wild-type glioma could increase NF- κ B activity and IL-6 levels, which can further activate EGFR.

In the tumors analyzed by Bredel et al. (2010), monoallelic, but not biallelic, loss of *NFKBIA* was observed. There are several potential interpretations of this result, again requiring further analysis. Loss of one copy of *NFKBIA* appears to be advantageous to the tumor, possibly for the reasons described above and/or for other reasons. However, it seems to be disadvantageous to lose both copies, as it was not observed in any of the datasets Bredel et al. utilized. Perhaps this speaks to the need to retain some degree of control and inducibility over the NF- κ B pathway (Figure 1). Another possibility is that I κ B α has roles besides its most well-studied function as an NF- κ B inhibitor and this is critical for oncogenesis or survival of cells of this particular lineage.

Deletions of *NFKBIA* in glioblastomas reported by Bredel et al. (2010) add to

the documented mutations in the NF- κ B pathway. Given the percentage of tumors with *NFKBIA* deletion and the impact on patient survival, this event seems to be significantly involved in glioblastoma development, potentially providing new targets for therapy. Future work requires further analysis of the downstream effects, particularly on the NF- κ B pathway. Comparison of *NFKBIA*-deleted and *EGFR*-amplified tumors will be important in determining whether these two alterations lead to a common phenotype or if they characterize two distinct subsets of glioblastoma. Potentially, the implication is that NF- κ B signaling could be centrally involved in all gliomas, although the mutations responsible may vary between subsets.

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PARP Inhibitors in Cancer Therapy: Promise, Progress, and Puzzles

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A recent article in the *New England Journal of Medicine* by O'Shaughnessy et al. provides evidence that a treatment strategy aimed at inducing DNA damage with chemotherapy while simultaneously disabling repair using a PARP inhibitor might offer hope for patients with a treatment-refractory form of breast cancer.

Many key genes inactivated in human cancer are involved in DNA damage and repair responses. Thus, specific DNA repair defects in tumor cells might be targeted selectively for therapeutic benefit in otherwise resistant malignancies. The emerging use of poly(ADP-ribose) polymerase (PARP) inhibitors in certain DNA repair-deficient cancers promises to fulfill this paradigm. Joyce O'Shaughnessy and colleagues recently demonstrated that treating patients with advanced

"triple-negative" breast cancers using the PARP inhibitor iniparib in combination with DNA-damaging chemotherapy increased tumor responses and prolonged patient survival compared with chemotherapy treatment alone (O'Shaughnessy et al., 2011). Like many important clinical advances however, this study leaves many unresolved questions, and addressing these will be critical to realizing the substantial promise of PARP inhibitors in cancer therapy.

PARPs catalyze the NAD⁺-dependent addition of poly(ADP-ribose) units to target proteins and regulate diverse cellular processes (Krishnakumar and Kraus, 2010). Most cellular PARP activity is attributable to PARP1, a ubiquitous and abundant nuclear protein that localizes to sites of DNA damage, leading to the recruitment of DNA repair proteins. Both PARP1 and PARP2 have been linked to base-excision repair, and *Parp1* null mice exhibit defective single-strand